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Female Myoblasts Can Participate in the Formation of a Male-specific Muscle in *Drosophila*

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ABSTRACT—In *Drosophila melanogaster*, a pair of dorsal longitudinal muscles in the fifth abdominal segment occurs in males but not in females. This male-specific muscle develops during metamorphosis. We examined how far the sexual identity of myoblast is involved in the formation of the male-specific muscle by transplantation of female myoblasts. A transformant strain which contains a 79B actin promoter fused to *Escherichia coli* β-galactosidase gene was used as a genetic reporter. A part of wing imaginal disc from the male or female donor transformant was transplanted into the abdomen of the male host (wild-type). After the hosts were allowed to develop till pharate adult or adult stage, the male-specific muscle of the host was examined whether it expressed the reporter gene or not. The expression of the reporter gene in the host's male-specific muscle was detected in both cases of donor's sex. The results indicate that myoblasts, independently of their sexual identity, can participate in the formation of the male-specific muscle. Therefore, the information of the sexual identity in the myoblasts itself is not the prerequisite for the formation of the male-specific muscle.

INTRODUCTION

Sexual dimorphism occurs in musculature and nervous system of most organisms, as well as in the external appearance. In *Drosophila melanogaster*, a pair of longitudinal muscles in the fifth abdominal segment occur in male adults but not in female ones [10, 14, 16]. The male-specific muscle, as well as other adult specific muscles, develops during metamorphosis [3, 6, 10]. Myoblasts which form adult abdominal muscles lie on branches of peripheral nerves and proliferate during larval and early pupal stages. They migrate out across the segment along the adult epidermis and fuse to make multinucleate myotubles. Examination of the formation processes of the male-specific muscle during metamorphosis suggested that the male-specific muscle would arise among the same pool of myoblasts to make other adult specific muscles in the 5th dorsal segment [10].

Differentiation of the male-specific muscle in males and the suppression of it in females is controlled by parts of sex-determining genes [19], although it is unknown that the action of the genes is autonomous in the muscle or non-autonomous. From the sex mosaic analyses, Lawrence and Johnston [16, 17] proposed that the formation is dependent on the innervation of the muscle by male-specific motoneurons but neither on a cell autonomous decision by sexual identity of the muscle fibers themselves nor on the sexual identity of the cuticular epidermal cells where the muscle inserts. In the present report, we examined directly the relationship between the sexual identity of myoblasts and the differentiation of the male-specific muscle by myoblast transplantation experiments. We used a transformant strain that contains a 79B actin promoter fused to the *Escherichia coli* β-galactosidase reporter gene, which is an useful differentiation maker of the male-specific muscle, because the 79B actin gene is expressed only in the male-specific muscle in the segment [5]. The transplantation experiments using this transformant showed that even female myoblasts, independently of the sexual identity, can participate in the formation of the male-specific muscle.

MATERIALS AND METHODS

Fly stocks

Flies were reared on cornmeal-yeast medium at 25°C under constant illumination. Males and females from the wild type strain *Canton-Special* (CS) were used for the analysis of normal development. The transformant line 72–3 which contains a 79B actin promoter fused to the *Escherichia coli* β-galactosidase reporter gene (*lacZ*) was used [5]. The resultant β-galactosidase is produced in muscles that activate the 79B actin gene [5]. For the staging of pupa, white pupae were collected and allowed to develop until desired stages at 25°C in a moisture chamber. Ages of these animals are given as hours after puparium formation (APF).

Immunohistochemistry

Mouse monoclonal antibody (Mab) 22C10 which stains the developing myoblasts and myotubles [10, 11] was used to follow the development of muscles during pupa. Immunohistochemical staining was done according to the methods by Usui and Kimura [20].

X-gal staining

X-gal staining for β-galactosidase activity of the reporter gene was performed using the method described by Hiromi *et al.* [12] with slight modifications [20].

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Transplantation experiments

Notum part of the wing disc contains many myoblasts (adepithelial cells) [2, 18]. The wing disc from the donor (72–3) strain at white pupal stage was dissected in phosphate buffered saline. The notum part of the disc was cut and transferred into the abdomen of the host (CS) strain at the same stage using a glass micropipette [7]. The myoblasts can be transplanted with the notum epithelial parts by this method [15]. The hosts are allowed to develop till pharate adult or adult stage. We could find the transplanted disc which had developed into adult tissue secreting cuitcle in the abdomen. The abdomen of the host flies was dissected and stained with X-gal, as described above.

RESULTS AND DISCUSSION

Figure 1 shows a comparison of adult musculature of dorsal abdomen between male and female. There are two types of muscles common to both sexes at least from the first to the 5th segments. One is dorsal oblique muscles of larval origin which persist throughout the metamorphosis. These muscles are temporally used for eclosion behavior and degenerate completely after eclosion [14]. The other is small dorsal longitudinal muscles which are newly formed during metamorphosis and continue to exist after eclosion. The male-specific muscle, a dorsal longitudinal muscle attaching the 5th abdominal tergite, can be distinguished from the others easily by its insertional position and length (Fig. 1).

Immunostaining with Mab22C10 enables us to follow the development of the male-specific muscle during the metamorphosis (Fig. 2). At 24 hr APF, the myoblasts which form adult abdominal muscles have been migrating out across the segment along the developing adult epidermis (Fig. 2A). Then the myoblasts fused to make multinucleate muscle precursors and at 32 hr APF they had aligned on the adult epidermis (Fig. 2B). At this stage, the male-specific muscle could not be recognized. At 36 hr APF, the myotubles had

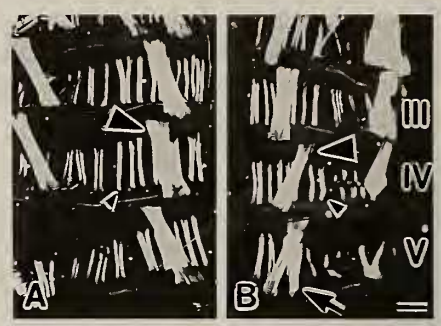

Fig. 1. Polarizing-light micrographs of adult musculature of the dorsal abdomen in female (A, left half) and male (B, right half) just after eclosion (CS strain). Males and females have muscles common to both sexes, larval persisting oblique muscles (large arrowheads) and dorsal longitudinal muscles (small arrowheads). A pair of extra thick longitudinal muscles (arrow) in the fifth abdominal segment occur only in male. The roman numerals in B indicate the abdominal segment. Bar in B, 100μm.

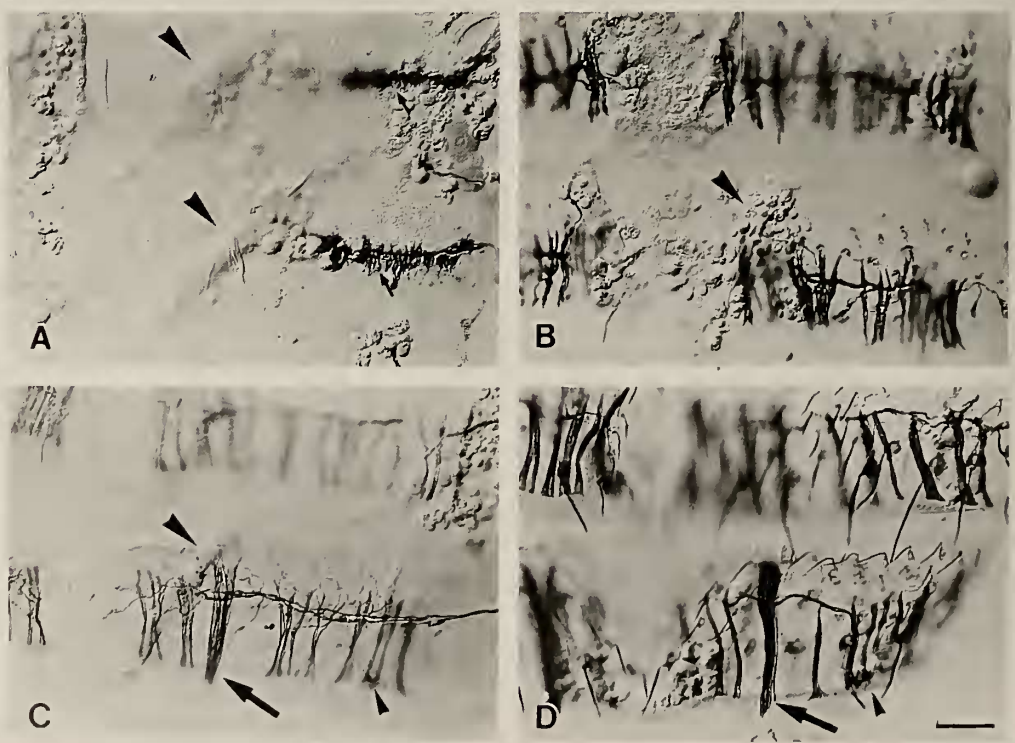

Fig. 2. Micrographs of the right half of the 4th and 5th segment of dorsal abdomen, showing adult muscle development during metamorphosis. Preparations from males of CS strain were stained immunohistochemically with Mab22C10. A, 24 h APF. B, 32 h APF. C, 36 h APF. D, 48 h APF. Large arrowheads, small arrowheads, large arrows and small arrows indicate larval persisting oblique muscles, dorsal longitudinal muscles, the male-specific muscles and myoblasts, respectively. Bar in D, 100μm.

formed and the male-specific muscle can be distinguished from the other small dorsal longitudinal muscles by its insertion position (Fig. 2C). At 48 hr, the muscles grow more (Fig. 2D).

The growth of the male-specific muscle was followed by the production of contractile protein, actin. Six actin genes are known in *Drosophila*, which show the tissue-specific and stage-specific expression patterns [9]. In the male-specific muscle, it is ascertained that the 79B actin gene is expressed at adult stage [5]. We examined the termporal pattern of the expression of the 79B actin gene (Fig. 3), using the transformant line 72–3 which contains a 79B actin promoter fused to *Escherichia coli* β-galactosidase reporter gene [5]. Till 50 hr APF, no expression of the reporter gene was observed in the male-specific muscle (Fig. 3A). The expression was observed at 54 hr APF (Fig. 3B) and became stronger as developed (Fig. 3C and D).

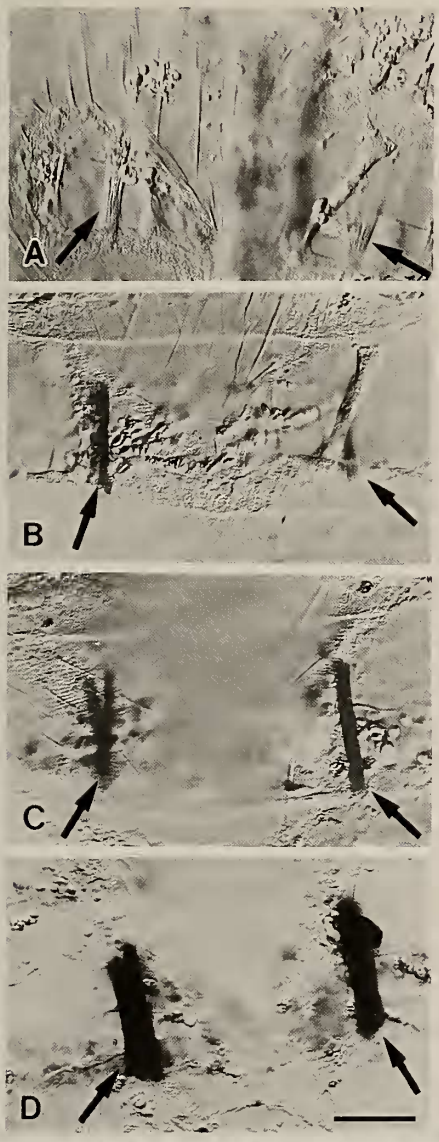

Fig. 3. Specific expression of the 79B actin gene in the male-specific muscle (arrows) during metamorphosis of the transformant pupae. The preparations were stained for β-galactosidase activity with X-gal. At 50 h APF, the reporter gene is not expressed (A). The expression is seen at 54 hr APF (B). Thereafter, the expression became stronger as developed (C, 60 h APF and D, 72 h APF). Bar in D, 100μm.

To understand how far the sexual identity of the myoblasts is involved in the formation of the male-specific muscle, we examined whether female myoblasts can participate in the formation of the male-specific muscle or not (Fig. 4). Firstly, myoblasts in the wing disc were transplanted from the male donor (72–3) into the abdomen of the male host (CS) of the same age. After the hosts were allowed to develop till pharate adult or adult stage, we examined whether the male-specific muscle of the host expressed the reporter gene derived from the male donor nucleus. Out of 9 transplantations, 6 hosts expressed the β-galactosidase in the male-specific muscle (Fig. 4B), indicating that the myoblasts in the wing disc can participate in the formation of the male-specific muscle. However, when myoblast in the wing disc were

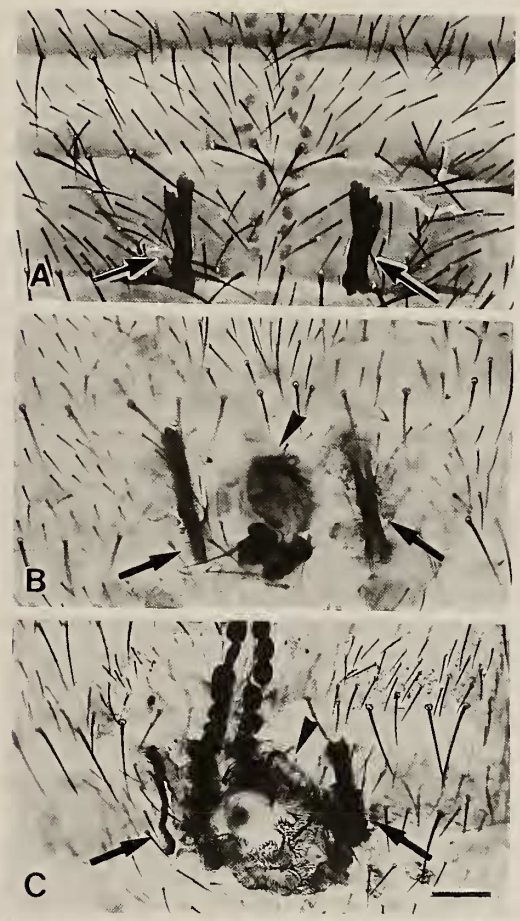

Fig. 4. Expression of the reporter gene transplanted from a transformant donor into a wild type host. The *lacZ* reporter gene expressed in the male-specific muscle of the donor male (A: control). The male-specific muscle of a male host expressed the reporter gene derived from transplanted myoblasts of either male transformant (B) or female one (C). Arrows show the male-specific muscle which expresses the *lacZ* reporter gene. Arrowheads in B and C indicate the transplanted disc which had developed into adult tissue secreting cuticle in the abdomen. Bar in C, 100μm.

transplanted from the male donor (72–3) into the abdomen of the female host (CS), the extra male-specific muscle nor the expression of the reporter gene were not seen in six female transplanted hosts. This indicates that the transplantation surgery does not affect the formation of the male-specific muscle. Next, we transplanted the myoblasts from 72–3 female into the CS male pupa. Out of 5, three hosts showed the expression of the reporter gene from female myoblasts in the male-specific muscle (Fig. 4C). This result indicates that even the female myoblasts can fuse into the developing male-specific muscle and that the expression of the actin gene derived from the female nucleus can be activated. The expression of the reporter gene derived from female myoblasts would be induced by some regulatory factors from host's male nuclei in the syncytium.

In *Drosophila*, determination of developmental fate in sexual dimorphic tissues is regulated through the activity of a cascade of several sex-determining genes [1]. It is shown that the differentiation of the male-specific muscle in males and the suppression of it in females are controlled by parts of sex-determining genes [19]. For examples, female flies mutant for allele of *Sex-lethal* or null alleles of *transformer* or *transformer-2* are converted into phenotypic males that formed the male-specific muscle [19]. Thus, wild-type products of the above genes act to prevent the differentiation of the male-specific muscle in female flies. Our results indicated that the cell autonomous decision of sexual identity in myoblasts themselves is not involved in the fusion process of myoblasts in the formation of the male-specific muscle. From the sex mosaic analyses, Lawrence and Johnston [16, 17] proposed that the formation of the male-specific muscle depends on the innervation of the muscle by male-specific motoneurons but neither on a cell autonomous decision by sexual identity of the muscles themselves nor on the sexual identity of the cuticular epidermal cells at the cuticle where the male-specific muscle inserts. In the embryo of *Drosophila*, the myogenesis occurs before the completion of the innervation by motoneurons [2, 13], indicating that the myogenesis itself occurs without innervation by neurons. Recently, it is shown that the innervation by motoneurons is prerequisite for the later differentiation of the muscles, for example localization of the transmitter receptor [4]. However, in the formation of adult muscles during metamorphosis, it is unknown how the neurons are involved in the processes of development of the muscles.

Our results of transplantation experiments also showed that the myoblasts in the wing disc, which normally form the muscles in the thorax [8], can participate in the formation of unusual muscles at the different position, the male-specific muscle in the abdomen. Similar results were also obtained by Lawrence and Brower [15]. These facts indicated that the specificity of muscles had not been committed in the myoblasts of the wing disc at least by white pupal stage. Commitment of the specificity of muscles may occur fairly late, around the time of innervation by motoneurons.

The male-specific muscle is an excellent material to investigate the relationship of innervating neurons and muscle differentiation. The identification of the male-specific motoneuron would help to reveal the mechanism of determination and differentiation of the sexually dimorphic musculature.

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REFERENCES

1 Baker BS (1989) Sex in flies: the splice of life. Nature 340: 521–524

2 Bate M (1990) The embryonic development of larval muscles in *Drosophila*. Development 110: 791–804

3 Bate M, Rushton E, Currie DA (1991) Cells with persistent *twist* expression are the embryonic precursors of adult muscles in *Drosophila*. Development 113: 79–89

4 Broadie K, Bate M (1993) Innervation directs receptor synthesis and localization in *Drosophila* embryo synaptogenesis. Nature 361: 350–353

5 Courchesne-Smith CL, Tobin SL (1989) Tissue-specific expression of the 79B actin gene during *Drosophila* devleopment. Dev. Biol 133: 313–321

6 Currie DA, Bate M (1991) The development of adult abdominal muscles in *Drosophila*: myoblasts express *twist* and are associated with nerves. Development 113: 91–102

7 Ephrussi B, Beadle GW (1936) A technique of transplantation for *Drosophila*. Am Nat 70: 218–225

8 Fernandes J, Bate M, Vijayraghavan K (1991) Development of the indirect flight muscles of *Drosophila*. Development 113: 67–77

9 Fyrberg EA, Mahaffey JW, Bond BJ, Davidson N (1983) Transcripts of the six *Drosophila* actin genes accumulate in a stage- and tissue-specific manner. Cell 33: 115–123

10 Gailey DA, Taylor BJ, Hall JC (1991) Elements of the *fruitless* locus regulate development of the muscle of Lawrence, a male-specific structure in the abdomen of *Drosophila melanogaster* adults. Development 113: 879–890

11 Hartenstein V, Posakony JW (1989) Development of adult sensilla on the wing and notum of *Drosophila*. Development 107: 389–405

12 Hiromi Y, Kuroiwa A, Gehring W (1985) Control elements of the *Drosophila* segmentation gene *fushi tarazu*. Cell 43: 603–613

13 Johansen J, Halpern ME, Keshishian H (1989) Axonal guidance and the development of muscle fiber-specific innervation in *Drosophila* embryos. J. Neurosci 9: 4318–4332

14 Kimura K-I, Truman JW (1990) Postmetamorphic cell death in the nervous and muscular systems of *Drosophila melanogaster*. J Neurosci 10: 403–411

15 Lawrence PA, Brower DL (1982) Myoblasts from *Drosophila* wing disks can contribute to developing muscles throughout the fly. Nature 295: 55–57

16 Lawrence PA, Johnston P (1984) The genetic specification of pattern in a *Drosophila* muscle. Cell 36: 775–782

17 Lawrence PA, Johnston P (1986) The muscle pattern of a segment of *Drosophila* may be determined by neurons and not by contributing myoblasts. Cell 45: 505–513

18 Poodry CA (1980) Imaginal discs: Morphology and development. In "The genetics and biology of *Drosophila* Vol 2d" Ed by M Ashburner and TRF Wright, Academic Press, New York, pp 407–441

19 Taylor BJ (1992) Differentiation of a male-specific muscle in *Drosophila melanogaster* does not require the sex-determining genes *doublesex* or *intersex*. Genetics 132: 179–191

20 Usui K, Kimura K-I (1992) Sensory mother cells are selected from among mitotically quiescent cluster of cells in the wing disc of *Drosophila*. Development 116: 601–610